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THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN CALIFORNIA. II *

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In the course of an investigation of certain phases of botulism, it was realized that the prevention of this intoxication should not rest with an attempt to destroy the spores, but should go further and determine, if possible, the natural habitat of this formidable species. Such an inquiry, conducted in California, was not only imperative on account of the rather frequent occurrence of human and animal botulismus following the consumption of vegetable products grown and packed in this state, but appeared most promising on account of the preliminary studies conducted by G. S. Burke.[1] This worker found the spores of B. botulinus on apparently sound fruits, vegetables and feeds collected at widely separated localities in California. The organism was not in any way associated with active decay, although on one occasion a strain was isolated from the feces of a hog which had ingested spoiled, toxic peas four months previous to the collection of the manure specimen. This latter observation coincided with the report of Kempner,[2] who recovered, in 1897, a strain of B. botulinus from the intestinal content of a "normal hog" and indicated that at least in a small percentage of instances the spores, and perhaps the vegetative stages of this anaerobe may persist as saprophytes in the intestinal canal of animals.

The successful demonstration of B. botulinus on fruits, vegetables, etc., by Burke immediately suggested the existence of this anaerobe in the soil. From the facts available, however, it was by no means clear whether B. botulinus occurred only in cultivated regions or whether the bacillus was a part of the common soil flora and multiplied under natural conditions in forest and mountain soil. The failures of van Ermengem[3] to isolate B. botulinus from various earth samples, such as garden soil, dirt from streets, canal slime, mud from ponds and rivers, etc., together with the relatively rare occurrence of botulismus

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[1] Jour. Bacteriol., 1919, 4, p. 541.

[2] Ztschr. f. Hyg. u. Infektionkr. 1897, 26, p. 482.

[3] Handbuch d. pathog. Mikroorganismen, 1912, 4, p. 936.

in certain states of the Union, indicated that the anaerobe was probably not omnipresent but occurred only in certain soils, probably due to accidental contaminations from heavily infected areas or possibly manure or animal excreta. These and similar considerations prompted an extensive cultural study of the telluric distribution of B. botulinus. Without an adequate knowledge of the biochemical activities and growth requirements of the bacillus at the time these studies were begun, it was naturally impossible to foresee the scope of the investigation. When the problem was approached, the prevailing idea of the intestinal habitat of sporulating anaerobes prompted the examination of cultivated and manured garden, field and pasture soils. Considerable attention was paid to the distribution of B. botulinus in various olive groves and on vegetables and fruits bought in the open market of various cities and towns of the state. The examination of control specimens and with it the frequent demonstration of B. botulinus in uncontaminated mountain soil, however, changed the original point of view so that, in addition to sampling field specimens in the vicinity of rural communities in which human or animal botulism had occurred, soil from uninhabitated, virgin territories was collected and cultures made. Simultaneously, numerous specimens of manure were studied, and the persistence of the spores of B. botulinus in the intestines of laboratory animals, as well as the factors responsible for the growth of the anaerobe in various earth specimens under laboratory conditions, was experimentally investigated. In order to verify some of the observations made in California, a broad investigation of soil and vegetable samples procured from every state of the Union, Canada, Hawaii, Alaska, China and Europe was subsequently made. This paper deals with the findings made on 607 specimens of soil and vegetables collected in California, and is mainly intended to serve as an introduction to the communications dealing with the distribution of the spores of B. botulinus in the United States.

COLLECTION AND STUDY OF SAMPLES

Soil specimens (200-500 gm. samples) were collected in small, carefully sterilized, sanitary tin containers (No. 1 cans). The lids of the containers were sealed and covered by sterile linen cloth. As a rule, samples of surface and subsurface soils (about 10 inches) were procured. Vegetables and fruits, as found in the open market or gardens, were packed in tight wooden boxes. Extreme precautions were taken to avoid extraneous contaminations in transit, as well as in the laboratory.

The writers are deeply indebted to Dr. J. C. Geiger, Epidemiologist of the U. S. Public Health Service, who carefully collected the majority of specimens on which these studies are based. A few samples were obtained by the Inspectors of the Food and Drug Department, California State Board of Health, by collaborators, by assistants and by veterinarians, who reported cases of "forage poisoning."

Adequate samples, varying from 10 to 100 gm. of soil, vegetables, etc., were treated according to the procedures outlined in a former paper.[4] All cultures were made with heated specimens in beef heart peptic digest liver broth. A number of the early cultures were incubated for from 4 to 6 weeks instead of 10 days, this shorter period was later adopted as a standard procedure. It is, therefore, not unlikely that on account of the deterioration of the toxin following the longer periods of incubation some of the specimens containing the spores of B. botulinus were not recognized. The cultures were always tested on guinea-pigs and the type of toxin determined on the same species of animal. About one-third of the toxic cultures produced characteristic symptoms of intoxication, but the nature of the toxin could not be ascertained by an antitoxin neutralization test. Sufficient experimental evidence has, however, been collected and presented in the first paper of this series, to support the conclusions that those soil or vegetable samples producing weakly toxic cultures contained only a few spores of B. botulinus. In a study dealing with the distribution of B. botulinus in nature, the findings pertaining to these weakly toxic specimens, even if they are not conclusively proved to be the result of B. botulinus by antitoxin tests, deserve recognition and are therefore included in the discussion.

EXPERIMENTAL DATA

The survey made in California during the years 1920 to 1922 covered the following phases of the problem: (1) A search for the spores in the soil or its products collected from gardens in widely separated localities in which human or animal botulism had occurred during the years 1919 to 1922; (2) systematic sampling of soil, vegetables and fruits from olive groves, spinach fields, which had furnished the raw materials, and later of products of packing plants, such as improperly sterilized canned products which had caused several outbreaks of human botulism; (3) culturing of fruits and vegetables bought in the open market; (4) toxicologic and cultural tests of feeds, hay, etc., supposed to have caused forage poisoning; (5) culturing of manure used in the fertilization of olive groves, vegetable gardens, etc.; (6) selective sampling and making of cultures of virgin soil collected in isolated areas of the Sierra Nevada, San Bernardino and Coast Range Mountains.

It is unnecessary to describe the various specimens and the time of the year during which the material was collected and cultures made. The results are summarized in table 1. For future reference, a presentation along geographic lines has been chosen and the findings previously reported by Dickson and Burke are included in the table. The survey is obviously not exhaustive, but the ubiquitous recognition of the spores in every section of the state made it unnecessary to extend the examination along broader telluric lines, although some of the early studies suggested a localization of the spores in certain districts and counties. This belief that the distribution of B. botulinus is confined to certain areas, towns, etc., followed the examination of a limited number of specimens and was readily disproved by the subsequent broader study of the problem. From a biologic point of view, intensive sampling of

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[4] Jour. Infect. Dis., 1922, 31, p. 501.

TABLE 1

RESULTS OF TESTS WITH SPECIMENS FROM VARIOUS COUNTIES IN CALIFORNIA

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Alameda County									
1	182C	Grizzly Peak, Berkeley Hills (1,759 ft.)	Virgin soil from new trail...	6	6	5	—	5	—	—	
2	33B	Oakland..............	Hay (2) and chicken manure	3	3	—	—	—	—	—	
3	15	Berkeley..............	One jar of spoiled string beans	1	2	2	—	2	—	—	
*	Report	Berkeley..............	Three jars of string beans and gizzards of 2 chickens	5	5	5	—	—	5	—	Isolated by E. C. Dickson
		Butte County									
4	4	Olive ranches, Oroville	Olives on trees, bird pecked and buried, hog manure	6	12	5	—	—	5	—	
5	81	Packing plant, Oroville	Decayed Sevillano and dried Mission olives	3	3	—	1 (Sevillano)	—	—	—	
6	81B	Packing plant, Oroville	Swelled cans of ripe pickled olives	3	3	1	—	—	1	—	Brine nontoxic
7	34	S. Ranch, Oroville.....	Soil (5), green olives and leaves (1)	6	15	—	—	—	—	—	Incubated for 12 weeks
8	35	O. Ranch, Oroville.....	Soil (6). green olives and leaves (1)	7	12	—	2 (soil)	—	—	—	Incubated for 12 weeks
9	36	P. Ranch, Oroville.....	Soil (7), green olives and leaves (2)	9	20	—	—	—	—	—	Incubated for 12 weeks
10	38	D. Ranch, Oroville.....	Soil (6). green olives and leaves (2)	8	18	—	—	—	—	—	Incubated for 12 weeks
11	39	E. Ranch, Oroville..... (1920)	Soil (6), manure (1), olives and leaves (2), fertilizer (1)	10	14	—	1 (soil)	—	—	—	One fertilizer positive; manure malignant edema
12	184	E. Ranch, Oroville..... (1921)	Soil, fertilized with manure	6	6	2	—	2	—	—	
13	52-54 IV	Chico..................	Hay suspected of causing forage poisoning	4	4	—	—	—	—	—	Extract nontoxic
14	68B	Chico..................	Hog manure...............	—	—	—	1	—	—	—	
15	62B	Chico..................	Mill run and hog tankage...	2	4	—	1	—	—	—	Incubated for 28 days
16	67	Chico..................	Soil.......................	16	16	—	4	—	—	—	
17	68	Biggs..................	Soil and decayed chickens (4) poisoned by spoiled corn	5	8	(chicken yard)	1 (crop) 1 (corn garden and burial ground)	2	—	—	
18	73	Durham...............	Hay, alfalfa, maize, calf-meal, rice	8	8	—	—	—	—	—	
19	100B	Packing plant, Oroville	Spoiled, pickled olives.......	9	9	—	1	—	—	—	

20	10	Colusa County Colusa	Decomposed hay (2), moldy beanstalk and plant (2), soil (2)	6	10	1 (bean plant)	2 (soil)	1	—	—	
21	20	Contra Costa County Pleasant Hill	Decomposed hay (forage poisoning)	—	—	—	—	—	—	—	Malignant edema
22	171	El Dorado County Lake Tahoe	Virgin soil	5	5	5	—	5	—	—	
23	76	Fresno County King's River Canyon	Spleen, intestine, liver, etc., of a horse	6	6	1 (intestines)	—	1	—	—	Forage poisoning (?)
24	76B	King's River Canyon	Soil from pasture, hill top	3	3	3	1	3	—	—	
25	76A	Fresno hills	Soil	3	3	—	—	—	—	—	
26	76B	Mountain valley	Virgin soil	3	3	1	—	1	—	—	
27	180A	Telden Pass	Virgin-soil	1	1	—	—	—	—	—	
28	180	Glenn County Orland	Hay and feed	2	2	—	—	—	—	—	
29	77	Humboldt County Eureka	Soil from vegetable gardens	8	8	—	—	—	—	—	Incubated for 28 days
30	77B	Eureka	Vegetables from open market	10	10	—	2 (parsnips)	—	—	—	
31	77B II	Eureka	Soil from vegetables	2	2	—	—	—	—	—	
32	180A	Inyo County Piute Pass	Virgin soil	1	1	—	—	—	—	—	5 gm. sample
33	66B	Kern County Bakersfield	Straw and burned hay (forage poisoning)	2	4	—	—	—	—	—	
34	5	Los Angeles County Long Beach, La Mosa Ranch	Moldy olives (3), soil (3)	6	6	—	—	—	—	—	
35	24A	52d St., Los Angeles	Garden soil, upper and lower layers	6	6	—	—	—	—	—	
36	26	Whittier	Hay (1), soil (1)	2	4	—	—	—	—	—	
37	29A	Whittier	Manure of sick horses	2	2	—	—	—	—	—	
38	54	Whittier	Hay (2), manure (2), corn (3)	7	7	4 (hay and manure)	—	2 (manure)	2 (hay)	—	
39	74A	Whittier	Hay (1), manure (2), barley (1)	4	4	—	1 (hay)	—	—	—	
40	50	Los Angeles	Cadaver of chicken	1	11	1 (9)	—	1 (9)	—	—	
41	50	Los Angeles	Chicken feed	2	2	—	—	—	—	—	
42	50	37th Ave., Los Angeles	Soil and string beans	2	2	—	—	—	—	—	See Human Reports 18 and 19
43	58	Sylmar Ranch	Soil	3	6	1	1	1	—	—	
44	58	Sylmar Ranch	Manure (2), Mission olives from trees	5	10	—	3 (olives, tree and manure)	—	—	—	
45	58	Sylmar Ranch	Soil	5	10	2	4	2	—	—	

TABLE 1—*Continued*

RESULTS OF TESTS WITH SPECIMENS FROM VARIOUS COUNTIES IN CALIFORNIA

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Los Angeles Co.—Cont.									
46	58	Sylmar Ranch.........	Olives from trees, leaves, etc.	4	8	1	2	—	1	—	
47	129B	51st St., Los Angeles...	Surface soil, chicken droppings, goat manure	4	4	—	2 (soil)	—	—	—	See Chicken Outbreak 21
48	129	Laguna Ranch, Los Angeles	Feed, manure, stagnant water, sewage (5)	9	9	—	4 (manure and beet pulp)	—	—	—	Bovine botulism suspected
49	134	New Hall Ranch.......	Soil (6), manure (2), decayed meat (1)	9	9	—	—	—	—	—	Malignant edema
50	135	New Hall Ranch.......	Pasture soil................	12	12	1	4	—	1	—	Five vibrion septique
51	139	Los Angeles...........	Barn feed and manure......	2	2	—	—	—	—	—	
52	181	Los Angeles...........	Corn husks and potatoes...	3	5	1	—	1	—	—	
53	224	Glendale..............	Three chickens and home canned corn	4	1 (6)	1 (6)	—	1 (6)	—	—	See Chicken Outbreak 107; isolated
54	136	Pasadena.............	Sewer farms, soil and sewage	4	4	—	2 (soil)	—	—	—	
55	196	Mount Lowe (6.100 ft.)	Soil (5), trail dust (1).......	6	6	2	1	2	—	—	Trail dust contaminated with horse feces contained B. tetani
		Marin County									
56	7	San Rafael............	Beanstalks (1), soil (2)......	3	5	—	—	—	—	—	See Human Report 15
57	8	San Rafael............	Beanstalks and soil.........	2	4	—	—	—	—	—	
58	25	San Rafael............	Soil........................	5	10	—	—	—	—	—	
59	K2	San Rafael............	Specimens of vegetables and fruits (cherries, beans, been root, asparagus, carrots)	12	25	3	3 (beans, vine, asparagus)	2 (asparagus)	1 (asparagus)	—	
60	181A	San Rafael............	Two fresh clams...........	2	2	2	—	2	—	—	
61	202	Mt. Tamalpais........	Virgin soil from Dipsea trail, Lone Pine, Forest Deer Park and Alpine trail	8	8	5	—	2 Lone Pine and Alpine trail 1 Forest Deer Park	—	2 Forest Deer Park and Dipsea	
		Mariposa County									
62	52B	Yosemite Valley.......	Virgin soil, Camp C.6, El Capitan Meadow and Ranger Station on Bridal Veil	4	8	2	—	2 (Camp 6)	—	—	
63	122	Yosemite Valley.......	Rockslide virgin soil, 1,000 feet above valley	4	8	3	1	3	—	—	
64	122	Yosemite Valley.......	Excavation in virgin soil 1 foot deep	6	6	6	—	6	—	—	
65	171	Gentry................	Virgin soil.................	8	8	2	—	1	1	—	

66	171	Mono County Mono Lake	Virgin soil	6	6	2	1	2	1	—	
67	42	Napa County Napa	Garden soil and vegetables.	13	13	—	1 (bean leaves)	—	—	—	
68	47	Calistoga	Garden soil, vegetables, hog and horse manure	10	10	—	—	—	—	—	
69	114	Napa	Soil from horse corral	2	2	—	—	—	—	—	
70	79B	Placer County Colfax	Garden soil (2), pasture soil (2)	4	4	—	—	—	—	—	Malignant edema; garden soil
71	172B	Roseville	Corral dirt and water (forage poisoning)	4	4	—	—	—	—	—	
72	183	Plumas County Feather River Canyon	Virgin soil	12	12	7	—	6	—	1	
73	135	Riverside County Riverside	Soil from orange groves, garden 1, Mt. Rubidoux	5	5	—	—	—	—	—	
*	Report	Sacramento County Sacramento	Jar of string beans, locally grown	—	—	—	—	—	1	—	1918, Mrs. G. S. Burke
*	Report	San Benito County Hollister	Water flies, slugs, sowbugs, earthworms, chicken manure, straw, dirt, mud, hog manure	—	—	1	—	—	1 (hog manure)	—	1918, Mrs. G. S. Burke
74	135B	San Bernardino County Cucumbo	Soil from gopher holes	2	2	—	—	—	—	—	
75	136	Sewer farms	Soil, top and deep	6	6	2	2	—	2	—	Spinach responsible for outbreak 63 grown in these farms
76	55	San Diego County San Diego	Soil, weeds and goat manure	6	8	4	—	4 (top soil and weeds)	—	—	Suspected botulism among goats
77	58	Escondido	Soil (3), potatoes (1), beans (1), hog manure (1)	6	13	2	—	2 (bean garden soil and hog manure)	—	—	
78	28	San Francisco County Golden Gate Park	Fertilized soil, deep and surface	5	10	—	—	—	—	—	
79	32	Golden Gate Park	Fertilized soil from nursery	5	10	—	—	—	—	—	
80	62C	San Joaquin County Stockton	Damp hay	1	3	—	—	—	—	—	
81	46	San Mateo County Menlo Park	Cadavers of chickens and soil	5	10	1	—	1	—	—	See Chicken Report 53
82	49	Menlo Park	Soil (8), bean leaves (1)	9	9	—	—	—	—	—	Incubated for 10 weeks

TABLE 1—*Continued*

RESULTS OF TESTS WITH SPECIMENS FROM VARIOUS COUNTIES IN CALIFORNIA

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Santa Barbara County									
83	9	Santa Maria..........	Straw (forage poisoning)...	1	2	1	—	—	1	—	
84	24	Santa Maria..........	Hay (1), soil (3)............	4	4	3	—	—	3 (soil and hay)	—	
85	127	Montecito.............	Peas, foliage and soil (2)...	4	4	3	—	2 (soil)	1 (foliage)	—	
86	127B	Santa Barbara........	Virgin soil and olives (San Carlo Ranch)	3	5	5	—	5 (olives, leaves and soil)	—	—	
87	202B	Montecito.............	Soil from Bishop Ranch....	1	1	1	—	—	—	1	
		Santa Clara County									
88	1	Saratoga.............	Chicken cadaver...........	1	10	1	—	1 (intestines)	—	—	
89	2	Saratoga.............	Garden soil, string beans...	2	12	2	—	2 (soil)	—	—	
90	40	Saratoga.............	Soil (7), fruits (4), vegetables (6), leaves (2), sewage	20	27	1	—	1 (soil, orchard)	—	—	
91	31	Morgan Hill..........	Fruits and vegetables......	5	8	1	1	1 (apricot)	—	—	
92	43	Los Gatos............	Soil (4), plums (2), bean leaves	7	9	—	4 (soil)	—	—	—	
93	61	San Jose..............	Vegetables from open market	15	15	5	2	5 (beans, celery, potato, turnips, lima beans)	—	—	
*	Report	San Jose..............	Chicken cadaver...........	—	—	—	—	1	—	—	Isolated by E. D. Dickson, see Report 1
*	Report	Palo Alto.......	Peas, beans, peaches, caterpillar, corn, cherries	—	27	2	—	2 (cherries)	—	—	Isolated by Mrs. G. S. Burke
		Santa Cruz County									
94	57	Boulder Creek.........	Garden soil and bean leaves	5	5	—	—	—	—	—	
		Shasta County									
95	70B	Redding...............	Virgin soil.................	6	6	—	1	—	—	—	
		Sonoma County									
96	219	Sebastopol-Healdsburg	Spoiled string beans, locally grown	1	1	1	—	1	—	—	
		Stanislaus County									
97	113B	Newman..............	Garden soil.................	6	6	6	—	6	—	—	
*	Report	Oakdale...............	Spotted leaves, pods, bush string beans, ants, spiders, bugs, grass hoppers	—	23	1	1 (spider, etc.)	—	1 (spotted bean leaf)	—	1918, Mrs. G. S. Burke
*	Report	Oakdale...............	Moldy hay..................	1	1	1	—	1	—	—	1918, Mrs. G. S. Burke

		Tehama County									
98	64	D. Ranch near Corning	Soil (4), bird pecked ripe olives	6	13	—	—	—	—	—	
		Tulare County									
99	51	Lindsay	Sh. Ranch soil (7), green olives (5), leaves (1)	13	13	1	1	—	1 (soil)	—	
100	51	Lindsay	G. Ranch soil (2), green olives (4)	4	4	1	—	1 (soil)	—	—	
101	51	Lindsay	Dr. Bruce soil (2), green olives (4)	6	6	—	—	—	—	—	
102	51	Lindsay	I. Ranch, oives and leaves	2	2	2	—	—	2 (leaves and green olives)	—	
103	51	Lindsay	Eck. Ranch soil (2), olives (2)	4	4	—	2 (soil) and olive)	—	—	—	
104	51	Lindsay	Eis. Ranch soil (1), olives (2), manure (2)	5	5	—	—	—	—	—	
105	51	Lindsay	Orange grove soil	2	2	—	—	—	—	—	
106	51	Packing plant, Lindsay	Scrapings pickling barrels (2), shipping barrels (1), olive dumps (1), conveyors (2), discarded olives (7)	13	13	1	1 (discarded olives)	1 (scrapings shipping barrel)	—	—	
		Tuolumne County									
107	171	Tioga Pass	Virgin soil	6	6	2	—	2	—	—	
		Ventura County									
108	41	Oxnard	Soil (1), hay (1), horse manure (1)	3	6	—	—	—	—	—	
		Yolo County									
109	80	Davis	Horse (2) and hog (2) feed, manure (4)	8	22	—	—	—	—	—	
110	87	Davis	Horse, hog and cow feed, manure	8	8	—	—	—	—	—	
		Unassigned—General Distribution									
111	22	San Francisco	Vegetables (2), fruits (1)	4	10	2	—	—	—	—	Toxin deteriorated before typing
112	1K	San Francisco	Fruits (11)	11	16	3	3 (turnip tops, plums)	2 (turnips, cherries)	1 (peaches)	—	
113	27	San Francisco	Vegetables	6	14	2	2 (radishes, peas)	—	2 (beets)	—	
114	30 and 33	San Francisco	Spinach, string beans, etc.	11	11	—	—	—	—	—	Incubated for 6 weeks
115	49B	San Francisco	Beet tops, corn, potatoes	4	6	2	1 (corn)	—	2 (beet tops)	—	

certain mountain areas deserves attention in the near future, but from a practical standpoint, at least, it is unnecessary to devote material and energy to an additional study of specimens collected in California. It has been definitely proved that the spores are widely distributed and may be found in soils and their products from any district or locality.

An analysis of the data presented in table 1 shows that 624 specimens have been examined by means of 894 cultures. These figures include also the samples of spoiled home canned products, tissues, etc., procured from animals after necropsies had been performed. Six hundred and seven specimens, representing samples of soil, vegetables, feeds, fruits and manure, were selected for further consideration and discussion; 179, or 29.4% of the specimens produced toxic cultures; 89 contained type A; 26 type B, and 4 type A and B B. botulinus toxins, while 60, or 9.8% of the cultures were not sufficiently toxic to furnish conclusive antitoxin neutralization tests. One hundred and nineteen, or 67.6% of the 179 toxic cultures were properly identified. Nearly three-fourths, or 74.8% of the typed cultures contained type A, showing clearly the predominance of this resistant type in the soil of California. Type B was found in 22.1% and a mixture of types A and B in 3.1% of the samples. The latter findings are important, as they definitely indicate that the 2 types of B. botulinus may coexist in the soil. The real biologic significance of the tabulated data was, however, not realized until the cultural findings were classified according to their origin. This classification is presented in chart 1.

For the sake of clearness, it is considered advisable to discuss the various columns separately.

1. *Virgin Soil Specimens.*—Only samples of soil collected from areas definitely known to be free from contaminations of animal excreta are included in this group; in fact, the majority of specimens represent earth of the Sierra Nevada or Coast Range Mountains. The first positive proof that B. botulinus exists in virgin soil was furnished by a number of soil specimens removed from a rockslide which occurred March 1, 1921, about 1,000 feet above the floor of the Yosemite Valley (El Capitan side, Big Oak flat). Samples were taken on April 20. Repeated cultural tests revealed the existence of spores of B. botulinus; the organism being isolated in pure culture on numerous occasions. Identical findings were made with soil collected from an excavation 4 feet deep in the heart of the valley, in which pollution with manure, etc., was absolutely excluded. These findings have subsequently been strengthened by the examination of soil specimens from the Sierra Nevada ridge near Tioga Pass, the Mono Lake and Lake Tahoe region, Feather River Canyon, etc. A total of 78, with 45, or 57.6%, toxic cultures has been studied. Forty-three samples of the latter cultures have been identified, and the predominance of type A has been definitely established. One sample, which originated from the vicinity of Gentry, Sierra foothills, Yosemite National Park, contained B. botulinus type B, while 2 specimens from Mount Tamalpais, near San Francisco, and 1 from the mouth of the Feather River Canyon contained mixtures of types A and B. Moreover, it is evident from the ease with which B. botulinus was isolated from the soil enrichment cultures and from the relatively low percentage of weakly toxic cultures that the spores are quite numerous in the virgin soil specimens. The evidence strongly supports the contention that B. botulinus is a common member of the soil flora in the Sierra Nevada and Coast Range Mountains.

2. *Garden, Orchard and Cultivated Field Soil Specimens.*—The samples included in this column originated from soil areas which were known to have

been fertilized either quite recently, or at least in the course of the last two to three years. In some instances, commercial fertilizer, but usually barnyard manure, had been employed. The majority of specimens were collected in olive groves and vegetable gardens. Recently a number of farms growing spinach and regularly flooded with sewage from Pasadena and other places have been tested. Fifty-nine cultures, or 26.9%, of the total 226 specimens were toxic. A relatively high percentage (40.6%) of the cultures was weakly toxic and could not be identified by antitoxin tests. These results are due to one or two conditions: either the number of spores of B. botulinus per gram of soil are few, or the concomitant anaerobes outgrow the toxin producers in the enrich-

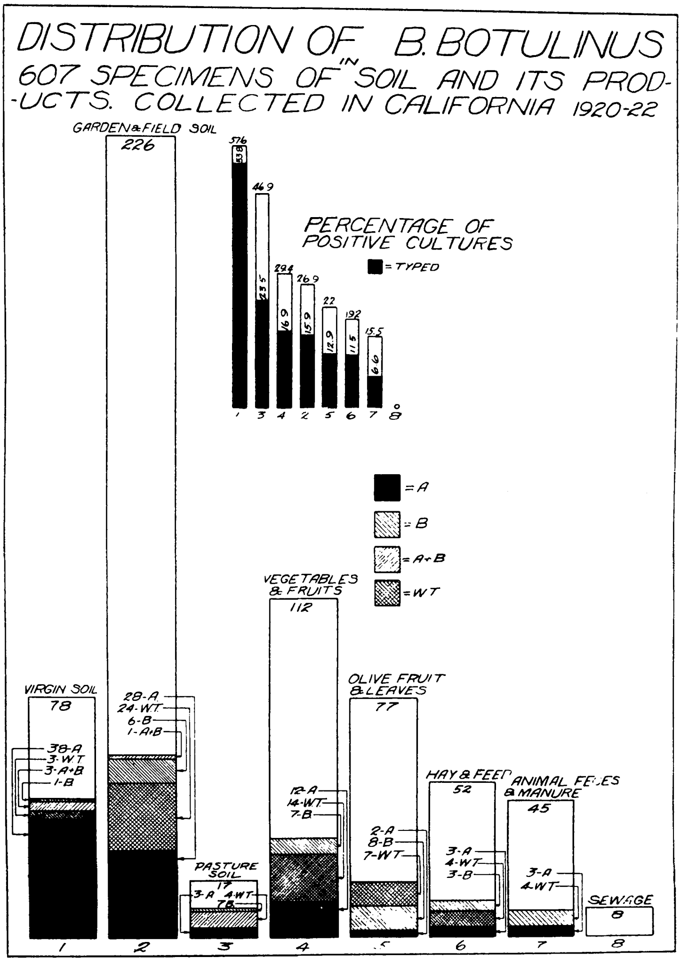

Chart 1

ment cultures. Type A was the predominant type, although type B appeared more frequently than in the virgin soil samples. The proportion of type A to type B for the virgin soil specimens was 38:1, while it shifted to 4.66:1 for the garden and cultivated field soil samples. The ranches from which the samples were taken are located in the Sacramento or San Joaquin Valley and receive their irrigation water from the Sierra Nevada Mountains. It is not unlikely that the fields are seeded with the type A spores from the water sources which are heavily infected with this organism. What happens to these spores can only be discussed after the various other columns have been carefully analyzed. It must, however, be concluded that the spores of B. botulinus are less frequently encountered in garden or cultivated field soil than in virgin mountain soil.

3. *Soil of Pastures.*—A small number of soils collected from pastures have been studied for the presence of B. botulinus. The specimens were collected from places on which horses or cattle had died from diseases diagnosed by local veterinarians as "forage poisoning." In every instance, the soil was markedly polluted with animal excreta, and it is significant that 9 of the 17 specimens produced "malignant edema" in guinea-pigs. Three of the 17 cultures contained type A, while one was neutralized by a type B antitoxin; 4 cultures could not be identified. Unfortunately, control tests of pasture soils not contaminated with animal manure were not made, and the limited number of specimens does not permit any definite conclusions.

4. *Vegetables and Fruits.*—This group of cultures was prepared with specimens either bought in the open market of San Francisco, San Jose (Santa Clara Valley), Los Angeles, Eureka (Humboldt County), or collected in gardens which had supplied the vegetables responsible as canned goods for cases of human or animal botulism. Cultures have been taken from potatoes, beets, asparagus, etc., heavily covered with soil, or fruits picked from the trees slightly contaminated with dust. In this connection it was noted that for example bird pecked cherries or bruised, partially spoiled apricots gave positive cultures more frequently than sound fruit; on one occasion, the soft, moldy blemish of an apricot gave a toxic culture, while the sound skin furnished a nontoxic culture. It is not unlikely that the spores of B. botulinus in the dust of the soil are fixed by the sticky sap of the bruised fruit. Thirty-three, or 29.4%, of the 122 specimens of vegetables and fruits produced toxic cultures. Fourteen, or 42.4%, of the cultures were weakly toxic and non-neutralizable, while 12 were neutralized by type A and 7 by type B antitoxins. The findings on the vegetables and fruits correspond in a general way with those made on the soil specimens collected in gardens and cultivated fields. The relatively high percentage of type B toxins has been repeatedly confirmed by the isolation of B. botulinus type B from the enrichment cultures. The vegetables, such as beets, asparagus, etc., bought in San Francisco were grown in truck gardens heavily fertilized with hog manure. Specimens of the fertilizer were tested for spores of B. botulinus with negative results. Soil products procured from plots recently planted with vegetables furnished enrichment cultures which contained type A toxins. Invariably the repeated examination of the soil demonstrated the same type. So far as the limited material permits of deductions, it is evident that the products of a soil which has been manured and cropped for many years more frequently contains the spores of B. botulinus type B than those of type A. Fruits and vegetables are excellently suited to demonstrate this distribution, probably on account of the relative minority of other proteolytic anaerobes which ordinarily destroy the toxins or overgrow B. botulinus present in specimens prepared from manured or fertilized soil.

5. *Olives, Foliage from Trees, Specimens from Olive Packing Plants.*—As a number of tragic human botulism outbreaks due to pickled ripe olives instigated the investigation on the distribution of B. botulinus in nature, it became necessary to follow the path of this organism from the olive grove to the packing plant. From the data summarized in column 5, it is evident that B. botulinus was readily demonstrated on olives. Not only were the leaves and fruits on the trees found to be contaminated, but the scrapings from the shipping barrels transporting the ripe olives from the groves to the cannery, as well as the refuse dumps composed of spoiled, discarded olives, supplied toxic cultures. Furthermore, several tins of ripe pickled olives, processed at low temperatures and unprocessed, so-called "bulk olives," gave pure cultures of B. botulinus type A. The frequent finding of type B spores only on the foliage and green fruits is closely connected with the predominance of this type in the manured soil of the ranches. This type is probably not very resistant against physical and chemical agents, rarely survives the pickling and preserving processes and has, therefore, not been found in the packed products.

Seventeen, or 22.0%, of the 77 olive specimens produced toxic cultures; the proportion of type A to type B was 1:4. The foliage and green olives collected from the trees furnished 10% weakly toxic enrichment cultures. From a practical standpoint, it is beyond question that B. botulinus is frequently carried on the ripe fruits into the shipping barrels, pickling vats and the packing plant. The question: Why did the organism under these prevailing conditions produce its toxin only in a very few nonsterile glass containers (probably 9 to 10 containers) is certainly not solved by the foregoing findings. It is not our purpose to enter into a theoretical discussion of a problem which was unfortunately not investigated when the conditions in the packing plants offered excellent opportunities for interesting disclosures. It is not unlikely that during and shortly after the war, the collection of ripe olives in the orchards was not conducted with a great deal of care. The raw product, heavily contaminated with soil, underwent further deterioration and the resistant spores were permitted to increase by prolonged storage in shipping barrels and pickling vats. The common practice of preserving the olives by heat not exceeding 212 F. produced in some containers an environment suitable for the growth of B. botulinus and its toxin. It is, however, now fully recognized that efficient sterilization of fresh and carefully handled ripe olives will prevent further outbreaks of botulismus.

6. *Hay, Straw and Animal Feeds.*—One-half of the specimens and cultures from which this column is computed originated from outbreaks of "forage poisoning," while the other half consisted of similar material collected in the vicinity of the outbreaks. Watery or saline extracts were usually found to be nontoxic for guinea-pigs, although 10, or 19.2%, of the 52 samples produced toxic cultures. Type A, as well as type B B. botulinus were demonstrated and isolated from the cultures. The percentage of positive cultures and the relative proportion of the two types of B. botulinus differed in no way from that established for the soil supplying the hay or feed. Furthermore, no differences were noted between the feeds suspected of having caused forage poisoning and those procured from sound haystacks or feeding troughs. The assumption that the finding of B. botulinus in the feed proves the botulismus nature of "forage poisoning" is not supported by the foregoing observations. In fact, it is shown that the mere cultural demonstration or isolation of B. botulinus in feeds, vegetables, etc., cannot be considered diagnostic of botulism.

7. *Manure and Fertilizers.*—Three, or 6.6%, of the 45 specimens contained the spores of B. botulinus type A. Four samples furnished weakly toxic enrich-

ment cultures. As a rule, large specimens varying from 100 to 500 gm. were employed. The low percentage of positive cultures is either due to an absence of the spores or the methods employed are unsuitable to detect the organism when present in relatively small numbers. It is not intended to discuss here the importance of some of the herbivorous animals as carriers or disseminators of B. botulinus spores, but the evidence strongly suggests that manure or fertilizers contribute relatively little to the pollution of the soil with this bacillus. On numerous occasions, parallel examinations of ranch soil and its fertilizing material revealed the presence of B. botulinus in the former and its absence in the latter. Moreover, it has been repeatedly pointed out that earth contaminated with animal feces is rarely infected, while mountain and forest soil in California are practically always infected with B. botulinus spores. In this respect the organism differs from its related species the tetanus bacillus. (See 54, Table 21.) It is not unlikely that occasionally the spores ingested with the feed are eliminated in the feces and in this manner herbivorous animals may act as distributors of the spores. Experimental studies to be presented by E. J. Easton in another paper confirm this conclusion and amplify the data outlined in this paragraph.

8. *Sewage.*—An extensive outbreak of botulism in chickens was investigated from both a pathologic and a bacteriologic standpoint. Vegetable and soil cultures revealed the fact that the garden in which the beans responsible for the fatalities were grown had been regularly fertilized by means of sewage and contained the spores of B. botulinus, type A. It was later found, however, that certain truck gardens furnishing spinach to canneries were constantly treated with sewage. Furthermore, a number of specimens of heated and unheated sewage were cultivated with entirely negative results, so that the evidence deduced from this outbreak among chickens can hardly be considered strikingly opposed to the previously established conception of the telluric origin of B. botulinus. It has been demonstrated in this laboratory that human excreta produce, as a rule, nontoxic enrichment cultures, irrespective of the fact that spores are frequently ingested on raw vegetables and fruits. Evidence is still lacking to prove that multiplication takes place in the human intestinal canal or in sewage and that the latter contributes to the contamination and pollution of the soil in certain districts or areas of this state.

In the light of the findings discussed under 7 and 8, it is evident that in California at least B. botulinus is not disseminated by animal excreta nor is the intestinal canal the natural habitat of this organism. This conclusion will be supported by data presented in subsequent papers, but it appears of interest to investigate briefly the distribution of the two types of B. botulinus as determined by this study. The predominance of type A in virgin soil and the occasional or relatively frequent occurrence of type B in manured gardens or ranch soil is sufficiently constant to suggest the existence of a biologic phenomenon. Data to be mentioned in a paper dealing with the morphologic and biochemical activities of 100 strains of B. botulinus indicate that the B types more often show variations in their cultural and serologic characteristics than the A types. In brief, it is believed that the former types are mutants of the latter. The biologic, physical and chemical influences encountered by the stable or fixed type A in cultivated and manured soil are probably responsible for the creation of a new adaptation type which can develop more readily in the new environment and as such preserve the genus Botulinus. The mutation is in this case favorable to the existence of the organism and enables it to multiply actively in an environment which is less suitable than the virgin, untilled soil. This interpretation of the origin of type B is quite in accordance with the observation that proteolytic anaerobes mutate more frequently than nonproteolytic types. One recalls in this connection the recently published paper of H. H. Heller[5] on mutations in the genus B. tetani and some of the studies of Löhnis[6] dealing with the life cycles of soil bacteria. It is obvious that this explanation deserves careful experimental investigation and is herewith offered as a working hypothesis for a field which has not only biologic but also some practical importance.

CONCLUSIONS

The examination of 624 specimens of soil, vegetables, fruits, feeds, manure and sewage collected in 36 counties of California and studied by means of 894 cultures definitely indicates that the spores of B. botulinus are very widely distributed. Approximately 30% of the samples produced toxic cultures. 74.8% of the identified toxins were neutralized by a type A, 22.1% by a type B and 3.1% by a polyvalent antitoxin.

The evidence strongly suggests that the natural habitat of B. botulinus is found in virgin mountain or forest soil. B. botulinus is also present in cultivated garden and field soils and their products. Vegetables and fruits bought in various cities and towns of California carry the spores of B. botulinus.

B. botulinus type B occurs predominantly in cultivated and manured soils and is probably a mutant of the fixed type A.

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[5] Jour. Infect. Dis., 1922, 30, p. 33.

[6] Jour. Agric. Res., 1916, 6, p. 675.